Journal of Chromatography A, 1217 (2010) 4383–4388

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Determination of acrolein and other carbonyls in cigarette smoke using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine

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ARTICLE INFO

Article history:
Received 17 February 2010
Received in revised form 14 April 2010
Accepted 20 April 2010
Available online 27 April 2010

Keywords:
Acrolein
Hydroquinone
2,4-Dinitrophenylhydrazine
High-performance liquid chromatography
Cigarette smoke

ABSTRACT

A new method for the determination of acrolein and other carbonyls in cigarette smoke using a dual cartridge system has been developed. Each cartridge consists of reagent-impregnated silica particles. The first contains hydroquinone (HQ) for the inhibition of acrolein polymerization, while the second contains 2,4-dinitrophenylhydrazine (DNPH) for the derivatization of carbonyls. Smoke samples were firstly drawn through the cartridge containing HQ-impregnated silica (HQ-silica) and then through the DNPH-impregnated silica (DNPH-silica). Acrolein in the sample was completely trapped in the first HQ-silica cartridge. Some other airborne carbonyls were also trapped by the HQ-silica, and those that pass through were trapped in the second DNPH-silica cartridge. Extraction was performed in the reverse direction to air sampling. When solvent was eluted through the dual-cartridges, excess DNPH was washed into the HQ bed where it reacted with acrolein and other trapped carbonyls to form the corresponding hydrazone derivatives. All of the hydrazones derived from airborne carbonyls were completely separated and measured using high-performance liquid chromatography. This HQ-DNPH-method can be applied for the determination of acrolein and other α,β-unsaturated aldehydes, such as crotonaldehyde, in cigarette smoke.

1. Introduction

Cigarette smoking causes 30% of all cancer deaths: the smoke contains more than 3500 chemicals and at least 50 of these are carcinogens [1,2]. Carbonyls, including acrolein (propenal), are among the compounds present at high levels. Long-term exposure to carbonyl compounds, such as formaldehyde and acetaldehyde, is known to increase the risk of asthma [3] and cancer [4]. Accurate carbonyl measurements are therefore important both for determining the formation mechanism of carbonyls and for evaluating their implication in human health. Acrolein, is not currently a suspected human carcinogen as, to date, no studies have been conducted to observe its carcinogenic effects on human cells. However, studies in rats have shown an increase in cancerous tumors from ingestion but not inhalation, and Feng et al. [5] have recently suggested a connection between acrolein in cigarette smoke and an increased risk of lung cancer. This emerging evidence suggests a need for an efficient technique for acrolein measurement.

For the analysis of carbonyl compounds including acrolein, their specific reaction with 2,4-dinitrophenylhydrazine (DNPH), forming the corresponding 2,4-dinitrophenylhydrazones, is one of the most important qualitative and quantitative methods in organic analysis. This was published by both Allen [6] and Brady [7] in the 1930s. The main advantage of the DNPH-method is the ability to analyze various aldehydes and ketones simultaneously in a complex mixture. Sampling can be performed using acidic solutions of DNPH in impingers [8–10] or with acidic solid sorbents using a DNPH-coated cartridge.

A number of cartridge devices containing solid sorbents coated with DNPH have previously been introduced for sampling aldehydes in air. The solid sorbents include XAD-2 [11,12], silica gel [13,14], glass beads [15], octadecylsilane bonded silica gel [16], Florisil [17], and glass fiber filters [18]. More recently, DNPH-coated silica gel has been widely used for a standard procedure by several national standardization bodies [19]. 2,4-Dinitrophenylhydrazone derivatives extracted from solid sorbent are usually separated by means of high-performance liquid chromatography (HPLC) and detected using UV spectrophotometry at 360 nm (depending on the absorption maximum of the hydrazones). However, for the analysis of α,β-unsaturated aldehydes such as acrolein and crotonaldehyde, numerous problems inherent in the methodology have been reported, including the instability of the acrolein DNPhydrazone (ACR-D) during collection and storage [20–23]. Contradictory data for the technique are found in the literature [20,24–27] and

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doi:10.1016/j.chroma.2010.04.056

severe problems have been observed in inter-laboratory comparisons [21].

Tejada was the first to describe the occurrence of chemical interference in the determination of acrolein using the DNPH-method [20]. During storage of samplers coated with acidified DNPH, the ACR-D peak disappeared and was replaced by an unknown reaction product in such a manner that the sum of the peak areas appeared to be invariant with time. Possanzini and DiPalo [24,25] identified two peaks for both acrolein and crotonaldehyde as syn- and anti-isomers. Risner and Martin [26], as well as Risner [27], traced the poor recovery observed during the determination of acrolein back to the formation of a dimer between two acrolein molecules (2-formyl-3,4-dihydro-2H-pyran) before derivatization. Progress has been made in resolving these limitations, such as using mass spectrometry instead of UV detection, but the instability of the ACR-D under the conditions necessary for collection from air had not yet been overcome.

Decomposition of ACR-D may be prevented by collecting acrolein away from the DNPH. In this study, an improved method which enables the determination of acrolein in addition to previously measurable carbonyls in air, and therefore cigarette smoke, using a dual cartridge system has been developed.

2. Experimental

2.1. Apparatus and reagents

The HPLC system (Shimadzu, Kyoto, Japan) used included two LC-20AD pumps, an SIL-20AC autosampler and an SPD M20A photo-diode array detector. The analytical column was an Ascentis Express RP-Amide, 2.7 μm particle size, 150 mm × 4.6 mm i.d. (Supelco Inc, Bellefonte, PA, USA). The column temperature was 40 °C and the injection volume was 10 μL. Two analytical conditions were adopted. The first was used in isocratic mode for rapid C_1–C_3 aldehyde derivative analysis and the mobile phase mixture was acetonitrile/water (60/40, v/v) containing 5 mmol/L ammonium acetate. The flow rate of the mobile phase was 0.6 mL/min. The second was used in gradient mode for C_1–C_{10} aldehyde derivative analysis. Solution A of the mobile phase mixture was acetonitrile/water (40/60, v/v) containing 5 mmol/L ammonium acetate and solution B was acetonitrile/water (75/25 v/v). HPLC elution was carried out with 100% A for 8 min, followed by a linear gradient from 100% A to 100% B in 37 min and then held for 15 min. The flow rate of the mobile phase was 0.7 mL/min.

The smoking machine model LM1/PLUS (Borgwaldt Technik GmbH, Hamburg, Germany) was used for collection of cigarette smoke. Air pump (SP-100 Dual GL Sciences Inc., Saitama, Japan) and wet gas meter (WS D-1A; Shinagawa Co., Tokyo, Japan) were used for the collection of air samples.

The water used for HPLC and sample preparation was deionized and purified using a Milli-Q Water System equipped with a UV lamp (Millipore, Bedford, MA, USA). The 2,4-dinitrophenylhydrazine hydrochloride (>98%) was obtained from Tokyo Kasei Co., Ltd., (Tokyo, Japan). The acetonitrile (HPLC grade, >99.9%), ethanol (>99.5), hydroquinone (>99%), phosphoric acid (85% solution in water), and ammonium acetate (99.999%) were from Sigma–Aldrich Inc., (St. Louis, MO, USA). Silica gel (spherical, 60/80 mesh, 120 Å mean pore size) was from AGC Si-Tech. Co., Ltd. (Fukuoka, Japan).

2.2. Preparation of a DNPH-impregnated silica cartridge (DNPH-cartridge) and a hydroquinone impregnated silica cartridge (HQ-cartridge)

Two types of DNPH-silica particles, for sampling low and high levels of carbonyls, were prepared. DNPH-silica: silica gel (50 g) was washed with water (3× 500 mL), methanol (2× 500 mL), and lastly acetonitrile (2× 500 mL). 2,4-Dinitrophenylhydrazine hydrochloride (0.25 g for low-level carbonyls and 1 g for high-level carbonyls) and phosphoric acid (0.5 mL for low-level carbonyls and 1 mL for high-level carbonyls) were dissolved in 200 mL acetonitrile. This solution was added to the washed silica gel (50 g), the mixture was stirred and the solvent was evaporated to dryness at 40 °C under vacuum on a rotary evaporator.

Hydroquinone (HQ)-silica: silica gel (50 g) was washed with water (3× 500 mL), methanol (2× 500 mL), and lastly acetonitrile (2× 500 mL). Then the solvent was completely evaporated to dryness at 100 °C for 30 min under vacuum on a rotary evaporator. After cooling to room temperature, acetonitrile (200 mL) was added to the washed silica gel. HQ (0.05 g) was dissolved in 50 mL acetonitrile. This solution was added to the washed silica gel, the mixture was stirred and the solvent was evaporated to dryness at 40 °C under vacuum on a rotary evaporator.

DNPH-silica (270 mg) and HQ-silica (270 mg) were packed into separate polyethylene cartridges (Rezorian tube, 1 mL, Supelco Inc., Bellefonte, PA) and stored in a refrigerator at 4 °C.

2.3. Collection and analysis of cigarette smoke and air sample

Before collecting a sample, an HQ-cartridge and a DNPH-cartridge were connected.

In the case of analyzing mainstream cigarette smoke, test cigarettes were prepared at 22 °C temperature and 60% humidity. Mainstream smoke constituents were collected under the conditions of 35 mL puff volume, 2-s puff duration, and 60-s puff interval according to ISO machine-smoking conditions [28]. A coupled cartridge was connected to the back of the filter, and cigarette smoke was drawn through the coupled cartridge from the HQ-cartridge to the DNPH-cartridge.

In the case of analyzing an air sample, air was drawn through a coupled cartridge from the HQ-cartridge to the DNPH-cartridge at flow rates of 0.1–1000 mL/min. After collection, the coupled cartridges were extracted with acetonitrile (containing 1% phosphoric acid) in the reverse direction to air sampling until the total volume of solution was 4.5 mL. After 10 min, the eluate solution was added with ethanol (0.5 mL) and was analyzed by HPLC. If the extraction was not performed immediately, the HQ-DNPH-cartridge set was decoupled and the individual cartridges were capped with stoppers.

3. Results and discussion

3.1. Decomposition of acrolein in the DNPH-cartridge

Acrolein standard gas (20 ppm) was drawn through DNPH-cartridges at a flow rate of 100 mL/min for 10 min. The DNPH-cartridges were then stored at 35 °C. At various times, the DNPH-cartridges were eluted with acetonitrile until the total volume of solution was 5 mL. The eluates were then immediately analyzed in isocratic mode using an HPLC instrument equipped with an autosampler set to 4 °C. Acrolein in air reacts with DNPH in the cartridge to form ACR-D. As DNPH-cartridge storage time increased, the peak of ACR-D decreased and unknown peaks appeared. Fig. 1 shows the chromatogram of the sample following 2.4 h storage at 35 °C.

ACR-D (λ_{max} = 372 nm) and four unknown peaks, a, b, c and d, were detected in the chromatogram. The maximum wavelengths of ACR-D, a, b, c and d were 373, 356, 350, 353 and 350 nm, respectively. Other unknown products may not have been extracted from the DNPH-cartridge. During the collection of acrolein, the intake side of DNPH-silica (about 1 mm thickness) changed color from yel-